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DATA EVALUATION REPORT

HED Doc # 011944

TAU-FLUVALINATE TECHNICAL

Study Type: ACUTE ORAL NEUROTOXICITY - RAT (81-8SS)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Task Order No. 94-47A

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Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Neurotoxicity – Rat (81-8ss)

TOX. CHEM. NO: 934

P.C. CODE.: 109302

MRID NO.: 434339-01

TEST MATERIAL: Tau-Fluvalinate Technical

SYNONYMS: N-[2-Chloro-4-(trifluoromethyl)-phenyl]-DL-valine cyano(3-phenoxyphenyl)-methyl ester; cyano(3-phenoxyphenyl)methyl N-[2-chloro-4-(trifluoromethyl)phenyl]-D-valinate; (RS)-α-cyano-3-phenoxybenzyl N-(2-chloro-α,α,α-trifluoro-*p*-tolyl)-D-valinate (IUPAC); ZR-3210; Mavrik

STUDY NUMBER: RCC 331683

SPONSOR: Sandoz Agro, Inc., 1300 East Touhy Avenue, Des Plaines, IL 60018

TESTING FACILITIES: RCC, Research & Consulting Company, Ltd., CH-4452 Itingen; Switzerland; BRL, Biological Research Laboratories, Ltd., Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland

TITLE OF REPORT: Determination of the Neurotoxic Potential of Tau-Fluvalinate Technical Following a 7-Day Oral Dosing (Gavage) in Rats

AUTHOR: A. Mahl

REPORT ISSUED: July 12, 1994

EXECUTIVE SUMMARY: In a short-term exposure oral neurotoxicity study (MRID 43433901), 10 male Wistar rats/dose group received 7 daily gavage doses of 0, 10 or 100 mg/kg body weight of Tau-Fluvalinate (87.7% a.i.) in corn oil (10 mL/kg). Due to severe toxicity, the high dose was discontinued and two additional groups of 10 male rats received 7 daily doses of 0 or 60 mg/kg. Functional observational battery tests (FOB) were conducted with clinical examinations pretreatment, on treatment days 1, 2, 4 and 7 and recovery days 7 and 14. Neural tissues were examined microscopically from 5 controls and 5 rats treated with 60 mg/kg after 7 days treatment and from the remaining control and 60 mg/kg animals after the 14-day recovery period. Motor activity was not quantitated.

At 10 mg/kg, a single incidence of ruffled fur (days 2 and 3) and salivation (day 2) and hyperalgesia (4/10 vs 6/20 controls) were noted. Food consumption was 83% of controls on days 1-4 of treatment. At 60 mg/kg, significantly decreased body weights were seen during treatment and recovery (86%, 77%, 74%, 84%, and 90% of controls on days 4 and 7 and on recovery days 1, 8, and 14, respectively). The food consumption was 31% and 26% of controls on days 1-4 and 4-7, respectively. Clinical/behavioral effects seen as early as Day 1 in all animals treated with 60 mg/kg but not in controls included salivation, ruffled fur, dyspnea, muscle spasms, and sedation. Other observations (observed in 30% to 90% of animals) included ataxia, coarse exertion tremor, hunched posture, gait abnormalities, serous reddish secretion from the nose, lids half closed, miosis, startle response hyperreaction, reduced grip strength (maximum, 42.2% compared to controls) and reduced rearing count. Also observed were fear, diarrhea, vibrissae reflex hyperreaction and hyperalgesia. The clinical/behavioral signs were transient and were not seen at the end of the recovery period. Peripheral nerve fiber degeneration was observed in animals treated with 60 mg/kg. The highest incidence and severity of nerve degeneration was seen in the sciatic nerve (minimal to moderate lesions in 4 animals compared with minimal lesions in 2 controls). Following the recovery, the incidence and severity of the lesions decreased. **The neurotoxicity LEL is 60 mg/kg, based on clinical signs of toxicity (observed as early as day 1) and peripheral nerve degeneration in male rats. The neurotoxicity NOEL is 10 mg/kg.**

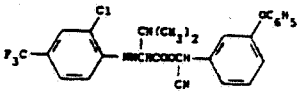
This study is classified as **Core-Supplementary (upgradable)** for an acute neurotoxicity study in rats and at this time does not satisfy the guideline requirement for 81-8SS. Numerous study deficiencies are noted in the Discussion section of this DER. The study may be upgraded upon submission of the following information: (1) information demonstrating sensitivity and reliability of laboratory neurotoxicity testing methods, and instrumentation and training of/proficiency of personnel; (2) data on analysis of dosing solutions for concentration, homogeneity and stability; (3) support for testing in only one sex (males).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test compound: Tau-Fluvalinate Technical

Purity: 87.7%
Description: viscous amber liquid, distinctive odor
Batch No.: 56816670
CAS No.: 69409-94-5
Contaminants: not specified
Structure:



2. Vehicle: Corn oil (Siegfried AG, CH-4800 Zofingen)

3. Test animals

Species: rats, males
Strain: HanIbm: WIST (SPF)
Age and weight at study initiation: 9 weeks; 207.2-231.4 g
Source: BRL, Biological Research Laboratories Ltd., Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland
Housing: individually in wire-mesh cages
Environmental conditions:
Temperature: 22 ± 3°C
Humidity: 40%-70%
Air changes: 10-15/hour
Photoperiod: 12 hour light/12 hour dark cycle
Acclimation period: 7 days

B. STUDY DESIGN

1. Animal assignment

Animals were randomly assigned to the test groups in Table 1. Only male rats were used because no sex-specific differences were observed in acute toxicity studies and males were found to be the more sensitive sex in long-term studies (information provided to study author by sponsor).

TABLE 1. ANIMAL ASSIGNMENT

Test group	Dose level (mg/kg)	No. assigned	
		A[a]	B[b]
1 Control	0	5	5
2 Low (LDT)	10	5	5
3 High (HDT)	100	5[c]	5[d]
4 Control	0	5	5
5 Mid (MDT)	60	5	5

Data taken from table on p. 20. MRID No. 434339-01.
[a]Termination after the 7-day treatment period.
[b]Termination after the 14-day recovery period.
[c]One rat died on day 4; two rats were treated from days 1 to 3 and sacrificed on day 4; two rats were treated from days 1 to 4 and observed through the recovery period.
[d]Four rats were treated from day 1 to 3 and sacrificed on day 4; one rat was treated from day 1 to 4 and observed through the recovery period.

2. Validation of test methods

Validation studies to demonstrate sensitivity and reliability of testing methods and instrumentation were not conducted. No positive control was used due to ethical reasons and because the test material, a pyrethroid, is assumed to exert specific effects on the nervous system.

3. Rationale for dose selection

The dose levels of 10 mg/kg/day (to produce minimal toxic effects) and 100 mg/kg/day (near lethal high dose) were based on the results of an acute range-finding study (RCC Project No. 331683) and of a pretest (RCC Project No. 335250) with repeated administration of the test material during seven consecutive days. A summary of the range-finding study is presented in the Appendix. Pretest data were not provided in the study report.

4. Preparation and analysis of dosing solutions

The test material solution in vehicle was prepared daily prior to dosing using a homogenizer. Homogeneity of test material in the vehicle was ensured during dosing by stirring the dilution for approximately 10 minutes using a magnetic stirrer. The dosing solution was administered by gavage at a volume of 10 mL corn oil/kg body

weight per treatment day. Although the study protocol indicated that dosing solutions would be analyzed for concentration, homogeneity, or stability, no data were included in the study report.

5. Diet

Pelleted standard Kliba 343 rat maintenance diet (batches No. 88/92 and 66/92; Kliba, Klingentalmühle AG, CH-4303 Kaiseraugst) and tap water *ad libitum.*

6. Statistical analysis

Statistical methods were used to analyze body weight, food consumption, and all ratios. If variables were assumed to follow a normal distribution, the Dunnett's test (many to one t-test) based on a pooled variance estimate was applied for comparison of treated and control groups of each sex. The Steel test (many-one rank test) was applied instead of the Dunnett's test when the data could not be assumed to follow a normal distribution. Group means were calculated for continuous data.

7. Signed and dated Quality Assurance (7/27/94) and Good Laboratory Practice (7/12/94) statements were present.

C. METHODS AND RESULTS

1. Clinical observations and mortality

Animals were observed for mortality or clinical signs of toxicity once daily during the acclimatization period; shortly before initiation of treatment; at 30 minutes and then hourly up to 9 hours after dosing on the first day of treatment; prior to and 1, 5, and 9 hours after each dosing from treatment days 2 to 7; and once daily during the recovery period. For clinical signs of toxicity, emphasis was placed on observations of disturbances of motor function (spontaneous motor activity, abnormal gait, ataxia, athetosis, choreoathetosis, paralysis, ventral or lateral recumbency, twitches, spasms, tremors, stereotypies, abnormal muscle tonus, catatonia), of autonomic function (dyspnea, palpebral closure, salivation, lacrimation, piloerection, pupil size, diarrhea, exophthalmos, skin color), and of general state of health (abnormal posture, sedation, aggressivity, fear, excitement, spontaneous vocalization or vocalization when touched).

Results – One rat in the high dose group (100 mg/kg) was found dead on treatment day 4 prior to dosing. Six additional rats of this group were sacrificed in extremis on treatment day 4 prior to dosing. The surviving three animals were put in recovery and observed to study termination.

Representative clinical observations are presented together with the FOB observations in Table 4 (the FOB examinations were performed together with the clinical observations on the days indicated below on p. 7 and were reported together in the study report).

2. Body weights

The body weight of each animal was recorded at the beginning of the acclimatization period, on treatment days 1, 4 and 7, and on recovery days 1, 7 and 14.

Results – Average body weights and body weight gains or losses are presented in Table 2. No statistically significant differences in body weights or body weight gains were noted in rats treated with 10 mg/kg. Animals treated with 60 or 100 mg/kg had significantly ($p \leq 0.01$) lower body weights on treatment days 4, 7 and recovery days 1 and 8. During the 7-day treatment, body weight of controls increased by about 10%, compared to weight losses of 10% (60 mg/kg) and 17% (100 mg/kg), and mean body weights at 60 and 100 mg/kg were less than their respective controls. Decreased body weights were related in part to the reduced food consumption during treatment at 60 and 100 mg/kg (see below). Although rats treated with 60 mg/kg gained weight from recovery days 8 to 14, the body weights at day 14 were still significantly ($p \leq 0.05$) lower than controls. The body weights of the 100 mg/kg group were comparable to controls on day 14 of recovery. The study author noted that the relevance of statistical results obtained in animals treated with 100 mg/kg is questionable because only 3 animals remained after day 4.

TABLE 2. AVERAGE BODY WEIGHTS AND BODY WEIGHT GAINS OR LOSSES (g)

Dose (test group)	Treatment day			Recovery day		
	1	4	7	1	8	14
0 mg/kg (group 1)	222.1	229.8 (+7.7)[a]	245.4 (+23.3)	246.8 (+24.7)	280.8 (+58.7)	303.5 (+81.4)
10 mg/kg (group 2)	220.9	222.5 (+1.6)	239.0 (+18.1)	242.2 (+21.3)	278.5 (+57.6)	305.9 (+85.0)
100 mg/kg[b] (group 3)	225.6	171.4** (-54.2)	202.1** (-23.5)	208.3** (-17.3)	257.1** (+31.5)	294.1 (+68.5)
0 mg/kg (group 4)	220.8	224.3 (+3.5)	238.1 (+17.3)	241.6 (+20.8)	265.4 (+44.6)	281.7 (+60.9)
60 mg/kg (group 5)	219.8	192.0** (-27.8)	182.3** (-37.5)	177.7** (-42.1)	224.1** (+4.3)	254.4* (+34.6)

Data taken from table on pp. 28 and 45-46. MRID No. 434339-01.
[a]Numbers in parenthesis are body weight gains or losses (in g) since start of treatment.
[b]Only 3 animals remained after day 3.
*Significantly different from control, $p \leq 0.05$ (Dunnett test).
**Significantly different from control. $p \leq 0.01$ (Dunnett test).

3. Food consumption

The food consumption was recorded at weekly intervals during the entire study period.

Results – Food consumption data are presented in Table 3. Compared with controls, the food consumption was significantly ($p \leq 0.01$) lower in animals treated with 10, 60, or 100 mg/kg during treatment days 1-4, and in rats treated with 60 mg/kg during treatment days 4-7. The decreases were marked at 60 and 100 mg/kg but slight at 10 mg/kg. During the recovery period, the food consumption of animals treated with the test material was comparable to controls.

TABLE 3. FOOD CONSUMPTION (g/ANIMAL/DAY)

Dose (test group)	Treatment days			Recovery days		
	1-4	4-7	1-7[a]	1-8	8-14	1-14[a]
0 mg/kg (group 1)	17.5	20.6	19.0	24.9	27.0	26.0
10 mg/kg (group 2)	14.5**	20.7	17.6	25.4	27.5	26.5
100 mg/kg[b] (group 3)	1.2**	16.9	9.0	24.2	27.5	25.8
0 mg/kg (group 4)	18.2	20.2	19.2	25.0	25.1	25.1
60 mg/kg (group 5)	5.6**	5.2**	5.4	23.1	25.0	24.0

Data taken from table on pp. 50-51. MRID No. 434339-01.
[a]Mean of means.
[b]Only 3 animals remained after day 3.
*Significantly different from control, $p \leq 0.05$ (Dunnett test).
**Significantly different from control, $p \leq 0.01$ (Dunnett test).

4. Functional observational battery (FOB)

An FOB was conducted together with clinical signs of toxicity (see p. 5 of DER) on acclimatization day 6, on treatment days 1, 2, 4 and 7 (prior to dosing), and on recovery days 7 and 14 (corresponding to study days 14 and 21). The study report did not indicate whether time of peak effect after dosing was determined prior to this study or whether animals were tested at peak effect. However, observations were conducted hourly on the first day for 9 hr post-dosing and were therefore adequately assessed according to the neurotoxicity guidelines. The examinations were performed in the following order:

a. Home cage observations together with clinical signs including ranking of the animals' reactivity (sensorimotor responses) with severity scores ranging from no reaction to hyperreaction (vibrissae reflex, pinna reflex, and startle response).

b. Observations in the test arena (animals observed for 1 minutes) together with clinical signs including arousal, level or state of alertness during observations of the unperturbed animal (rearing count, exploratory behavior) and autonomic functions of the unperturbed animal (count of fecal boli and urine pools).

c. Observations outside the home and test arena including salivation; lacrimation; discharge from the nose; fur and skin appearance; body and limb tonus; pupil size; corneal reflex; pupil reaction to light; behavior in relation to the observer (fear, aggressivity, ease of handling, vocalization during handling); pain response; visual placing ability; climbing ability; forelimb grip strength; and body temperature (for ethical reasons, landing foot splay examinations were not performed).

Results - FOB parameters and clinical signs of toxicity are shown in Table 4 (data for the two control groups are combined, n = 20). Additional observations not shown in the table included the following: muscle twitching of the head, abnormal posture, chromodacryorrhea (one animal each) and deep yellow urine (two animals) at 60 mg/kg; ptosis, ventral recumbency, waddling gait, negative visual placing ability, and chewing movements (one animal) and deep respiration and pale feces (two animals each) at 100 mg/kg.

At 10 mg/kg, the only effects noted included a slightly higher incidence of vocalization when touched and hyperalgesia compared with controls. One animal showed salivation on treatment day 2 and ruffled fur on treatment days 2 and 3 but not at later times. The severity of these effects was ranked as slight.

At 60 and 100 mg/kg, the first clinical signs, salivation, ruffled fur, and vocalization when touched, were noted 2 hours after the first dose and more or less continuously during the entire treatment period. Salivation and ruffled fur on occasion received highest severity scores. Effects indicative of impaired motor function included coarse exertion tremor, muscle spasms, gait abnormalities, ataxia, muscular hypertonus on the neck and back, muscular twitchings in the head region, and abnormal posture. Impaired motor function was confirmed by impaired climbing ability and reduced grip strength (with a maximum impairment of 71.2% at 100 mg/kg and 42.2% at 60 mg/kg compared to controls). The most pronounced effects on motor function disturbances in animals at 60 mg/kg were seen between treatment days 2 and 5. AT 100 mg/kg, ataxia was reported in one animal on day 1 only. There was a slight tendency of adaptation during the last two treatment days. Body temperature and grip strength measurements on treatment day 7 also suggested slight adaptation. Rearing counts were decreased at 60 and 100 mg/kg compared with the corresponding controls.

Disturbances of the autonomic function including salivation, dyspnea, serous reddish secretion from the nose, ruffled fur, and miosis were observed at a higher incidence and degree of intensity 5 and 9 hours after dosing. These signs were also seen occasionally prior to dosing suggesting persisting effects or intervals of alternating intensity of effects during the treatment day. Reduced fecal boli and deep yellow urine were attributed to diminished body water content caused by severe salivation.

TABLE 4. FUNCTIONAL OBSERVATIONAL BATTERY

Observations	0 mg/kg	10 mg/kg	60 mg/kg	100 mg/kg
Acclimatization period[a]				
No clinical/behavioral signs	14/20[b]	9/10	9/10	7/10
Vibrissae reflex hyperreaction	5	1	0	2
Startle response hyperreaction	2	0	1	1
Hyperalgesia	2	0	1	0
Treatment period				
No clinical/behavioral signs	9/20	5/10	0/10	0/10
Sedation	0	0	10 (1-7)[c]	10 (1-4)
Fear	1 (4)	0	3 (2-4)	2 (1,2,3)
Ataxia	0	0	3 (3,4)	1 (1)
Spasms paws neck	 0 0	 0 0	 10 (2-7) 1 (7)	 2 (2,3) 1 (4)
Coarse exertion tremor	0	0	4 (2,3,6)	5 (2,3)
Muscular hypotonus, back and neck	0	0	0	3 (3,4)
Hunched posture	0	0	9 (2-7)	10 (2-4)[d]
Abnormal gait	0	0	6 (3,4,5-7)	10 (2,3,4)
Dyspnea	0	0	10 (2-7)	10 (1-4)
Ruffled fur	0	1 (2,3)	10 (1-7)	10 (1-4)
Diarrhea	1 (7)	0	3 (2,4,6)	4 (2,3,4)
Feces containing mucus	0	0	1 (6)	3 (3,4)
Reduced size of fecal boli	0	0	1 (7)	1 (3)
Salivation	0	1 (2)	10 (1-7)	10 (1-4)
Serous reddish secretion from nose	0	0	8 (2-7)	9 (2,3,4)
Lids half closed	0	0	7 (2-7)	8 (2,3)
Miosis	0	0	5 (1,2,7)	4 (2,3,4)
Vocalization when touched	9 (1,2,4-7)	5 (1-4,7)	10 (1-7)	8 (1-4)
Vibrissae reflex hyperreaction	1	1	4 (2,4,7)	9 (2,4)

TABLE 4. Continued

Vibrissae reflex hyporeaction	0	0	1 (2)	3 (4)
Startle response hyperreaction	0	0	4 (2,4,7)	9 (2,4)
Pinna reflex hyperreaction	0	0	0	2 (2)
Hyperalgesia	6 (2,4,7)	4 (2,4,7)	9 (2,4,7)	9 (2,4)
Analgesia	0	0	0	3 (4)
Impaired climbing ability	0	0	1 (2,7)	7 (2,4)
Reduced grip strength	0	0	8 (2,4,7)	10 (2,4)
Reduced rearing count	0	0	6 (2,4)	9 (2,4)
Strongly decreased body temperature	0	0	2 (2,4)	8 (2,4)
Recovery period				
No clinical/behavioral signs	7/10	4/5	0/5	0/5
Vocalization when touched	3 (1,2,5,7,13,14)	1 (5)	3 (1,2,4)	3 (1-9,13,14)
Hyperalgesia[e]	1 (7)	0	1 (7)	2 (7)
Sedation	0	0	3 (1)	3 (5)
Hunched posture	0	0	3 (1,2)	0
Abnormal gait	0	0	1 (1)	1 (5)
Ruffled fur	0	0	5 (1-3)	3 (1-5)
Miosis	0	0	1 (1)	0
Serous reddish secretion from nose	0	0	0	2 (5)
Pale feces	0	0	0	1 (5)
Reduced grip strength	0	0	0	2 (7)

Data taken from pp. 29-32 and 59-60, MRID No. 434339-01.
[a]Examined on day 6 of acclimatization period.
[b]Number of animals/group affected. Animals from the two controls groups are combined.
[c]Numbers in parentheses are days on which observations were made.
[d]Study report (p. 31) indicated first observation on day 1 but this was not supported by the individual animal data
[e]During treatment period examined only on days 2, 4, and 7; during recovery period on days 7 and 14.

Effects on sensorimotor function indicating an increased sensitivity to external stimuli were observed throughout dosing and included increased vibrissae reflex, pain response, startle response, and response to touch. In the more severely affected animals, however, reduced reactions during testing of the vibrissae and pinna reflex and pain and startle responses were noted. Body temperature was significantly decreased at 60 mg/kg ($p < 0.05$, below 34°C in 1 animal) and 100 mg/kg ($p < 0.01$, below 32°C in 3 animals) at days 2 and 4 of treatment.

Ruffled fur, hunched posture, and vocalization when touched were still seen during the first few recovery days in animals treated with 60 mg/kg, but by recovery day 5, no clinical signs of toxicity were observed. At 100 mg/kg, ruffled fur was noted up to day 8 of recovery and reduced grip strength values were measured until recovery day 7. Compared with controls, a higher incidence of vocalization when touched persisted until study termination.

5. Motor and locomotor activity

Motor and locomotor activity were not measured.

6. Clinical chemistry

Clinical chemistry parameters were not analyzed.

7. Sacrifice/necropsy/neurohistopathology -

a. Animal sacrifice and processing of tissues - All animals were examined grossly for abnormalities. The group 3 animals (100 mg/kg) that died or were sacrificed were also examined. Five animals of each group (two controls, 10 and 60 mg/kg and 3 remaining animals at 100 mg/kg) were sacrificed on study day 8 after termination of the 7-day treatment period. The remaining animals were necropsied on study day 22 after termination of the recovery period. All animals were anesthetized by intraperitoneal injection of Narcoren at a dose of 2.0 mL/kg body weight (equivalent to 320 mg sodium pentobarbitone/kg body weight), perfused with physiological saline containing heparin followed by Karnovsky's fixative (preparation of glutaraldehyde/paraformaldehyde). Brain, spinal cord, sciatic nerve, sural nerve, and tibial nerve samples were stored in Karnovsky's fixative.

Preserved tissues from group 5 (60 mg/kg) and the corresponding control group (group 4) were processed for histopathologic examination. The brain and spinal cord were embedded in paraffin wax and stained with hematoxylin and eosin and specimens of the peripheral nerves (sciatic, sural, and tibial; whole nerves) were embedded in methacrylate and stained with Toluidine-blue. Histopathologic examinations were not performed for group 3 (100 mg/kg) since treatment was discontinued on day 4 and for group 2 (10 mg/kg) since no behavioral abnormalities were observed.

The following nerve tissues and brain regions were examined microscopically:

Brain		Spinal Cord		Peripheral nerves	
X	Cerebellum	X	Cervical	X	Sciatic nerve
X	Forebrain	X	Thoracic	X	Tibial nerve
X	Midbrain	X	Lumbar		Peroneal nerve
X	Pons		Gasserian ganglion	X	Sural nerve
X	Medulla oblongata		Dorsal root ganglion		Optic nerve
	Olfactory region		Cauda equina	Other	
			Nerve roots		
					Eye
					Gastrocnemius

Results -

a. Gross observations - No treatment-related gross lesions were noted at necropsy in the animal that died on study or in animals sacrificed *in extremis* or at the scheduled date.

b. Neurohistopathology - No microscopic lesions were observed in the brain or spinal cord of animals treated with 60 mg/kg of the test material or in their corresponding controls.

In the peripheral nervous system, an increased incidence of nerve fiber degeneration was seen in rats treated with 60 mg/kg compared with the spontaneous findings in the control group (Table 5). Nerve degeneration was characterized by digestion chambers, histiocytic cells and/or broken or absent axons or infolded myelin sheaths and was seen in affected animals in more than one nerve. The highest incidence and severity (ranging from minimal to moderate) of nerve degeneration was seen in the sciatic nerve affecting four animals compared with minimal lesions seen in two controls. In the sural and tibial nerves, one animal each had moderate and slight lesions and three animals had minimal lesions compared with minimal lesions seen in two controls.

Following the recovery period, fewer and generally less severe nerve lesions were seen than at the end of treatment. With the exception of lesions in the left sciatic nerve (where all treated animals were affected), there were no clear differences between control and treated animals at the end of recovery.

TABLE 5. INCIDENCE AND SEVERITY OF PERIPHERAL NERVE DEGENERATION[a]

Peripheral Nerve	Control		60 mg/kg	
	Term.[b]	Rec.[c]	Term.	Rec.
Sciatic nerve, left				
minimal	1	3	1	4
slight	0	0	3	1
Sciatic nerve, right				
minimal	2	2	2	0
slight	0	0	1	0
moderate	0	0	1	0
Sural nerve, left				
minimal	1	3	3	1
moderate	0	0	1	0
Sural nerve, right				
minimal	2	1	2	1
Tibial nerve, left				
minimal	0	0	2	1
Tibial nerve, right				
minimal	0	1	2	0
slight	0	0	1	0

Data taken from pp. 156-161, MRID No. 434339-01.
[a]Five rats/group examined
[b]Terminal sacrifice group
[c]Recovery group

D. DISCUSSION

Treatment with 60 mg/kg of Tau-Fluvalinate caused an increased incidence and severity of nerve fiber degeneration affecting the sciatic, sural, and tibial nerves in animals sacrificed at the end of the treatment. The recovery animals appeared to show at least partial recovery based on decreased mean severity of these lesions, but the number and severity of animals affected by sciatic degeneration was slightly greater than controls (4 minimal, 1 slight at 60 mg/kg vs. 3 minimal in controls). Except for sciatic nerve lesions, the nerve fiber degeneration was no longer apparent after the recovery period. No histopathologic lesions were detected in the CNS tissues (sections of the brain and spinal cord). Peripheral nerve degeneration demonstrates a neurotoxic potential of the test material at 60 mg/kg, the dose which also produced clinical signs of toxicity and behavioral effects as well as a severely impaired general state of health (decreased body weights and food consumption, hunched posture, half closed lids and sedation). The study author noted that the animals' poor state of health may have contributed considerably to the effects on nervous system function and structural integrity.

A low incidence of clinical signs of neurotoxicity (ruffled fur, salivation, vocalization, and hyperalgesia) was seen at 10 mg/kg. Severity of these effects was graded as minimal, did not persist throughout treatment and could not be attributed unequivocally to treatment with the test material because of the mild severity, subjectivity of the endpoints and observation in only one animal. At 60 and 100 mg/kg, severe effects were recorded in the FOB and daily observations of clinical signs of toxicity. The affected parameters reflected impaired motor function, autonomic function, and sensorimotor function. Sedation, ruffled fur, miosis, and salivation were seen as early as day 1 after dosing. All of these effects were completely reversible by day 5 of the recovery period.

Because effects were minimal and observed in only one animal, a neurotoxicity NOEL of 10 mg/kg for male rats appears to be justified. Furthermore, this NOEL may represent a conservative endpoint for acute neurotoxicity since it is based on repeated dosing. The neurotoxicity LEL is 60 mg/kg based on pronounced clinical signs of neurotoxicity and peripheral nerve degeneration.

The doses were selected from an acute neurotoxicity range-finding study in which rats were administered single gavage doses of 150, 200 or 250 mg/kg of Tau-Fluvalinate. Administration of the test material produced marked to severe clinical signs of neurotoxicity at all dose levels and reduced body weight gain during the first week after dosing. Although repeated dosing with 100 mg/kg/day in the main study had to be discontinued due to severe toxicity by day 4, selection of 100 mg/kg as the initial high dose appeared to be justified.

E. STUDY DEFICIENCIES

There were several major deficiencies in the conduct of this study according to the Agency neurotoxicity testing guidelines which are listed below. The following deficiencies should be addressed in order to upgrade the study to acceptable:

- Validation studies to demonstrate sensitivity and reliability of laboratory neurotoxicity testing methods and instrumentation in accordance with EPA Neurotoxicity Testing Guidelines, Addendum 10, were not provided: adequate demonstration of the training and proficiency of personnel involved in testing and of sensitivity of methods and equipment should be submitted;
- Data on analysis of dosing solutions for concentration, homogeneity or stability were not provided: this information should be provided;
- Only one sex (males) was tested. According to the study author, no sex-specific differences were apparent in previously conducted acute toxicity studies and males were found to be more sensitive than females in long-term studies; however, details of these studies were not provided: information supporting this conclusion should be submitted, including demonstration that the incidence of clinical signs of toxicity following acute exposure is similar for both sexes.

The following deficiencies are also noted and discussed, but do not require additional attention by the study author:

- Motor activity was not measured: this is considered a major data section of neurotoxicity studies. However, given the numerous indications of effects on gait and activity observed at 60 and 100 mg/kg, it may be assumed that motor activity is likely to be affected at those dose levels. It is unlikely that any motor activity would be observed at 10 mg/kg, particularly after a single dose;
- Microscopic examination of nervous system tissues was not performed at the low dose of 10 mg/kg even though lesions were noted at 60 and 100 mg/kg; because of the lack of clinical signs at 10 mg/kg and the relatively low severity grade of the lesions at 60 mg/kg, this information is not required to upgrade the study;
- Some neural tissues such as dorsal root ganglia and cauda equina were not examined; since pyrethroids primarily affect peripheral nerves additional data is not required;
- The test material was administered over a 7-day period instead of as a single acute dose in order to maintain higher blood levels over an extended period that was expected to produce nerve tissue damage and behavioral effects consistent with neurotoxicity induced by pyrethroids. The study author noted that pyrethroid insecticides are known to produce morphological changes in the peripheral nerves of rats when given at lethal or near lethal doses [H.P.M. Vijverberg and J. van den Bercken. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Critical Reviews in Toxicology 21:89-104 (1990)]. Lower doses, however, do not cause these effects (W.N. Aldridge. An assessment of the toxicological properties of pyrethroids and their neurotoxicity. Critical Reviews in Toxicology 21:297-316)]. The effects on peripheral nerves are considered secondary to the primary action of pyrethroids, i.e., effects on sodium channels. Although the study demonstrated that multiple dosing with the test material causes peripheral nerve damage, it should be noted that 7-day dosing may be inappropriate for determining neuropathology from acute exposure: Since the NOEL of 10 mg/kg is expected to provide a conservative neurotoxicity endpoint for an acute exposure, the study may be used for that purpose. Daily clinical/FOB observations may be used to evaluate single exposure effects;
- Clinical signs of toxicity and FOB observations were not presented separately; however, this omission does not detract from the validity of the study.

Classification: Supplementary (upgradable)

APPENDIX A

DOSE SELECTION STUDY

Study Type: Acute oral neurotoxicity range-finding in rats
Test Material: Tau-Fluvalinate Technical
Testing Facilities: RCC, Research & Consulting Company, Ltd., CH-4452 Itingen, Switzerland; BRL, Biological Research Laboratories, Ltd., Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland
Study Title: Acute Oral Toxicity (Dose-Range-Finding) Study with Tau-Fluvalinate Technical in Rats
Author: A. Mahl
Project no.: 331683
Date: February 2, 1993

Methods:

Test animals: male HanIbm: WIST (SPF) rats, 9 weeks old, weight 204.8-229.8 g
Group size: 3 animals/dose
Treatment Protocol: single doses of 150, 200 or 250 mg/kg administered in corn oil (10 ml/kg) by gavage, followed by 14-day observation period. Body weights were determined pretest and days 1, 4, 8, and 15. Clinical signs were observed pretest, during the first hour and then hourly up to 7 hours after application, and then once daily from observation day 2 to 15. Necropsies were performed at the end of the observation period.

Results:

Mortality: None

Body weight gain: During the first three days after dosing, each of the animals in the three dose groups lost weight (12 to 35.5 g). On observation days 8 and 15, the animals had gained weight within the normal range of variation.

Clinical observations: The following clinical signs, many graded as marked to severe, were noted in all test groups during the first observation week: aggressive behavior, excitement, increased spontaneous activity, sedation, spasms, ataxia, abnormal posture, dyspnea, ruffled fur, diarrhea, salivation, lids half closed, hyperalgesia, spontaneous vocalization, vocalization when touched, pinna reflex and startle response, and impaired climbing ability. In addition, fear was observed at 150 mg/kg; sedation, serous red secretion from nose, and hyporeaction during testing of pinna reflex at 200 and 250 mg/kg; and ataxia and hyperreaction during testing of vibrissae reflex at 250 mg/kg. During the second observation week, aggressive behavior, vocalization when touched, and hyperreaction during testing of the vibrissae reflex were occasionally observed in individual animals. Additionally, hyperalgesia was noted at 250 mg/kg. Recovery from treatment was noted on day 13 at 150 mg/kg, day 9 at 200 mg/kg and day 11 at 250 mg/kg.

Macroscopic pathology: No abnormalities were noted at necropsy.

Dose selection: Based on the results of this study, 100 mg/kg was found to be appropriate as the high dose for the acute neurotoxicity study.

Core classification: Not applicable; range-finding study.